INTRODUCED LESSER CELANDINE (*RANUNCULUS FICARIA*, RANUNCULACEAE) AND ITS PUTATIVE SUBSPECIES IN THE UNITED STATES: A MORPHOMETRIC ANALYSIS

Angela R. Post[1]

Department of Horticultural Science
North Carolina State University
Raleigh, North Carolina 27695-7609, U.S.A.

Alexander Krings

Herbarium, Department of Plant Biology
North Carolina State University
Raleigh, North Carolina 27695-7612, U.S.A.
Alexander_Krings@ncsu.edu

Wade A. Wall

Department of Plant Biology
North Carolina State University
Raleigh, North Carolina 27695-7612, U.S.A.

Joseph C. Neal

Department of Horticultural Science
North Carolina State University
Raleigh, North Carolina 27695-7609, U.S.A.

ABSTRACT

Ranunculus ficaria is native to Europe, but was introduced to the United States by at least the 19th century as a garden ornamental. Following introduction, the species escaped from cultivation. Because, in contrast to European floristic treatments, previous North American floristic treatments had not emphasized subspecific recognition, our objectives for this study were to determine if and how many morphologically recognizable entities within *R. ficaria* occur in the United States, evaluate to what extent such entities correspond to the subspecific concepts followed in Europe, and analyze the distributions, habitats, and rates of spread of each entity. To meet our objectives, we conducted a morphometric analysis based on study of 319 specimens from forty-seven herbaria. The combined results indicate the presence of five entities reasonably referable to the subspecies accepted in Europe. If one accepts subspecies as incompletely diverged lineages, one would expect a limited amount of overlap of operational taxonomic units (OTUs) as seen in our Principal Coordinates Analysis and Principal Components Analysis results, as well as incompletely sorted OTUs as seen in our cluster and classification tree analyses. Based on our current understanding, all five subspecies occur in the United States. They are best adapted to moist sites, exhibit overlapping distributions, and appear to be spreading at similar rates.

RESUMEN

Ranunculus ficaria es nativa de Europa, pero fue introducida en los Estados Unidos como ornamental en el siglo XIX. Después de la introducción, la especie escapó de cultivo. Porque, en comparación con los tratamientos florísticos europeos, los norteamericanos no habían enfatizado en el reconocimiento subespecífico, nuestros objetivos en este estudio fueron determinar si existen y cuantas entidades reconocibles morfológicamente de *R. ficaria* se dan en los Estados Unidos, evaluar en que medida tales entidades corresponden al concepto de subespecie que se sigue en Europa, y analizar las distribuciones, hábitats, y tasas de expansión de cada entidad. Para lograr nuestros objetivos, realizamos un análisis morfométrico basado en el estudio de 319 especímenes de cuarenta y siete herbarios. Los resultados combinados indican la presencia de cinco entidades razonablemente referibles a las subespecies aceptadas en Europa. Si se aceptan subespecies como líneas divergentes incompletas, se podría esperar una cantidad limitada de solapamiento en las unidades taxonómicas operativas (OTUs) como aparecen en nuestro Análisis de Coordenadas Principales y Análisis de Componentes Principales, así como OTUs incompletamente ordenados como se ven en nuestros análisis de clusters y árboles de clasificación. Basados en nuestro conocimiento presente, las cinco subespecies están en los Estados Unidos. Están mejor adaptadas a lugares húmedos, muestran distribuciones que se solapan, y parece que se extienden a velocidades semejantes.

INTRODUCTION

Ranunculus ficaria L. (Ranunculaceae) is native to Europe (Tutin 1964; Taylor & Markham 1978; Sell 1994; Whittemore 1997), but was introduced to the United States (U.S.) through the garden ornamental trade for its showy flowers (Bailey 1935). It was collected with certainty in the U.S. in 1867 (Philadelphia County, Pennsylvania, *Burke s.n.*, PH) and required 141 years to "spread" from Philadelphia, Pennsylvania to Fort Worth, Texas (*Nesom FW08-1*, BRIT, MO, NCSC, NCU, TEX)—the southernmost extent of the current known distribution (Nesom 2008).

[1]**Present address: Department of Plant Biology, Cornell University, Ithaca, NY 14853, arp232@cornell.edu**

In Europe, five subspecies of *Ranunculus ficaria* are recognized (Tutin 1964; Sell 1994), whose "ecology and [...] distribution appear to overlap, but tend to be different" (Sell 1994). *Flora Europaea* currently recognizes: (1) *Ranunculus ficaria* subsp. *ficaria* from western Europe, eastward to southern Italy, (2) subsp. *bulbilifer* Lambinon from northern and central Europe, extending to Spain, Albania and east-central Russia, (3) subsp. *calthifolius* (Reichenb.) Arcangeli from south-central and eastern Europe, (4) subsp. *chrysocephalus* P.D. Sell from Greece and Crete, and (5) subsp. *ficariiformis* (F.W. Schwartz) Rouy & Fouc from southern Europe. Plants are known to be diploid (2n=16), triploid (2n=24), or tetraploid (2n=32). Diploids have been referred to subspecies *calthifolius* and *ficaria*, and tetraploids to *bulbilifer, chrysocephalus*, and *ficariiformis* (Greilhuber 1974; Sell 1994). Triploids may represent putative hybrids and have apparently been collected from widely separated localities in Europe (Marchant & Brighton 1974; Sell 1994). Sell (1994) suggested that a large proportion of the pollen of triploids, as well as the tetraploid subsp. *bulbilifer*, is non-viable and few seeds are set. Pollen from diploids and the large-flowered tetraploids (i.e., *chrysocephalus* and *ficariiformis*) is apparently viable and many achenes are produced (Sell 1994). Two subspecies are capable of producing bulbils in their leaf axils: *bulbilifer* and *ficariiformis*. Subspecies *bulbilifer* tends to exhibit globose bulbils and subsp. *ficariiformis* produces ellipsoid bulbils (Sell 1994).

In contrast to European accounts, North American floristic treatments either recognized no subspecific taxa in *R. ficaria* (Fernald 1950; Gleason 1952; Gleason & Cronquist 1963; Whittemore 1997) or only variety *bulbifera* Marsden-Jones (=subsp. *bulbilifer* Lambinon; Magee & Ahles 1999). However, the recent discoveries of entities putatively referable to subsp. *ficariiformis* in North Carolina (Krings et al. 2005) and Texas (Nesom 2008; reported as subsp. *bulbilifer*, but with ellipsoid bulbils and flower dimensions within the range of *ficariiformis*) caused us to question whether additional subspecies may be present in North America that have not been previously recorded and if so, whether these differed in their distributions, habitats, and rates of spread. Because prior North American treatments did not emphasize subspecific recognition and as subspecies are not uniformly accepted, our objectives were to: (1) determine if and how many morphologically recognizable entities within *R. ficaria* occur in the United States, (2) evaluate to what extent such entities correspond to the subspecific concepts followed in Europe (based on the work of Sell 1994), and (3) analyze the distributions, habitats, and rates of spread of each entity.

METHODS

Distribution and habitat information were recorded from 319 herbarium specimens, requested from the following forty-seven herbaria based on previous literature reports (Benson 1942; Bell 1945; Gleason & Cronquist 1991; Whittemore 1997): A, AUA, BALT, BH, BKL, BRIT, CONN, CU, DOV, F, FLAS, GA, GH, HNH, ILLS, KE, LGO, LSU, MARY, MASS, MICH, MISS, MO, MOR, MSC, MT, MU, NA, NCSC, NCU, NHA, NY, OS, OSC, PH, POM, TENN, TEX, UNA, US, USF, USCH, VDB, VPI, WTU, WVA, Y (Appendix A). Herbarium label data recorded for each specimen included collector name, collector number, date, habitat, and county and state of collection. Specimens lacking information were excluded from the study.

Collection localities were classified into the following nine habitat classes: (1) adjacent to a water source, (2) disturbed areas, (3) dry woods, (4) fields, (5) horticultural, (6) lawns, (7) lowlands, (8) moist areas, and (9) roadsides. A specimen was classified as adjacent to a water source if it was collected along the banks of a river, stream, or pond. Moist areas were defined as moist or alluvial woods, swamp or bog areas, and other moist shade. The horticultural class was defined as being collected in a nursery or garden under cultivation. Lowlands were defined as low or depressed areas where moisture level was not mentioned on the label. Fields were defined as any open grassy area not maintained as a lawn and in full sun such as pastures and meadows.

To determine if and how many morphologically recognizable entities within *R. ficaria* occur in the United States, each herbarium sheet was treated as an operational taxonomic unit (OTU) for data capture and pertinent subsequent analyses. To evaluate to what extent such entities correspond to the subspecific concepts followed in Europe, each specimen was determined to subspecies following the key constructed by Sell (1994):

1. Leaf blades to 8 × 9 cm; petioles to 28 cm; flowers to 60 mm diam; achenes 5.0 × 3.5 mm.
   2. Stems rather robust, but straggling; bulbils present in leaf axils after flowering ____ subsp. **ficariiformis**
   2. Stems robust and erect; without bulbils in leaf axils after flowering ____ subsp. **chrysocephalus**
1. Leaf blades to 4 × 4 cm; petioles to 15 cm; flowers to 40 mm diam; achenes to 3.5 × 2.2 mm.
   3. Leaves crowded at the base with few on short stems ____ subsp. **calthifolius**
   3. Leaves less crowded at base and more numerous on the elongate stems.
      4. Bulbils not present in leaf axils after flowering; achenes well developed ____ subsp. **ficaria**
      4. Bulbils present in leaf axils after flowering; achenes poorly developed ____ subsp. **bulbilifer**

Sell (1994) recognized the difficulty in identifying *Ranunculus ficaria* to the subspecific level, noting that specimens should be examined throughout the growing season for positive identifications. We agree with him and others (Whittemore 1997; Nesom 2008) that identification can be challenging and recognize the impact identifications have on analysis results. However, we feel reasonably confident in our subspecies assignments due in part to the quality of specimens, which facilitated taxon assignment, as well as our own field observations and phenological analyses. Of the 319 herbarium specimens examined 232 had at least a month and year date on the label and 90% of these were collected mid-April through June. Another 5% were collected in the last week of March and the remaining 5% were collected in January through mid-March. Based on date of collection, the majority of specimens examined in this study were collected late enough in the spring that they would exhibit bulbils if they were genetically capable of producing them.

Using a digital caliper, the following morphological measurements were taken from each OTU: (1) leaf length from up to ten leaves, (2) leaf width from up to ten leaves, (3) petiole length from up to ten leaves (using same leaves measured for length and width), (4) petal length from up to ten flowers, (5) petal width from up to ten flowers, (6) achene length of all achenes present, (7) achene width for all achenes present. The presence or absence of bulbils was also recorded.

Quantitative and qualitative data were studied jointly and separately. Statistic analyses, including ANOVAs and post-hoc tests (Tukey's HSD), were carried out in the statistics package R (Ihaka & Gentleman 1996; R Foundation for Statistical Computing 2008). Prior to multivariate analysis, we tested all quantitative univariate variables using the Shapiro-Willks normality test and subsequently $\log_{10}$ transformed them to minimize the influence of allometry on the results (Dufrêne et al. 1991; Almeida-Pinheiro de Carvalho et al. 2004; Pimentel et al. 2007). Gower's dissimilarity coefficient for mixed data was used to quantify resemblances between OTUs (Gower 1971). The relationships between OTUs were subsequently explored with both hierarchical agglomerative cluster analyses and principal coordinates analyses (PCoA) using the complete set of characters. Three different sorting algorithms were used to help distinguish between data-dependent and potential method-dependent differences in results, following Dickinson & Phipps (1985) and Pimentel et al. (2007): single linkage, complete linkage, and average linkage (UPGMA; Sneath & Sokal 1973).

Quantitative characters were also analyzed separately using Principal Components Analysis (PCA). PCA is an objective, correlation-based technique that allows the variance in quantitative characters to be considered simultaneously and the subsequent visualization of dispersion patterns in a number of dimensions that explain the greatest amount of variance (Sargent et al. 2004; Joly & Bruneau 2007; Pimentel et al. 2007). A Kaiser-Meyer-Olkin (KMO) test was performed prior to the PCA to assess the suitability of the data for multivariate analysis (see also Almeida-Pinheiro de Carvalho et al. 2004; Pimentel et al. 2007).

Classification trees were employed to help identify specific morphological characters that could reliably separate the OTU groups corresponding to the five putative subspecies sensu Sell (1994). Classification trees divide datasets with pre-assigned group membership into increasingly homogeneous subsets in tree-like fashion based on the included morphological traits. Recovery proceeds until the groups obtained are pure or until a dividing threshold is achieved (Joly & Bruneau 2007). For the classification tree, all morphological characters were included and quantitative data was not log transformed prior to analysis.

The distribution of *R. ficaria* was mapped based on herbarium specimens and using ArcGIS 9.2 (ESRI 2004). The rate of spread for each subspecies was determined based on the number of counties each was present in during each decade from 1860 to the present. These data were analyzed using proc mixed in SAS

9.1.3 with a critical value of 0.05 (SAS Institute 2002–2005). Note that we use "spread" in a broad sense, as we suspect that not all new county records are the result of physical movement of propagules from established parental plants, but that many may reflect novel introduction events.

RESULTS

Descriptive statistics

Table 1 provides non-transformed means and standard deviations for the seven quantitative characters measured for each group of OTUs assignable to one of five putative subspecies sensu Sell (1994) and summarizes the results of the one-way ANOVAs and subsequent post-hoc tests (Tukey's HSD) on $\log_{10}$ transformed data (see superscripts). Figure 1 exhibits box-plots showing the distribution of non-transformed quantitative measurements taken by OTU group. Significant differences in the means for each of the five groups of OTUs assigned to the subspecies sensu Sell (1994) for all seven characters were found (Table 1). Post-hoc tests (Tukey's HSD) to determine which sample means differed from which others showed that means of leaf length and petal width of the OTU group assignable to subsp. *chrysocephalus* differed significantly ($p<0.05$) from the respective means of the groups of OTUs assignable to the four other subspecies (Table 1). OTUs assignable to the diploid subsp. *calthifolius* and *ficaria* differed significantly ($p<0.05$) in mean leaf length, mean leaf width, and mean petiole length (Table 1). They did not differ significantly in mean petal length, mean petal width, mean achene length, or mean achene width. OTUs assignable to the tetraploid subsp. *bulbilifer*, *chrysocephalus*, and *ficariiformis* differed significantly ($p<0.05$) from one another in mean leaf length and mean petal width. Subspecies *bulbilifer* differed significantly ($p<0.05$) from both subsp. *chrysocephalus* and *ficariiformis* in mean leaf width, mean petiole length, and mean petal length. Subspecies *chrysocephalus* differed significantly ($p<0.05$) from subsp. *bulbilifer* in mean achene length and width, but subsp. *ficariiformis* differed neither from subsp. *chrysocephalus* nor *bulbilifer* in these characters (Table 1).

Cluster analyses

In all three cluster analyses—average, complete, and single linkage—OTUs were resolved into two large divisions, these corresponding to (1) the bulbil bearing taxa: subsp. *bulbilifer* and subsp. *ficariiformis* sensu Sell (1994) and, (2) the non-bulbil bearing taxa: subsp. *calthifolius*, subsp. *chrysocephalus*, and subsp. *ficaria* sensu Sell (1994; Fig. 2). Within these two divisions, the topologies resulting from the three different algorithms differed notably only for those resulting from single linkage. Within the *bulbilifer/ficariiformis* division, average linkage recovered a cluster predominantly composed of OTUs referable to subsp. *ficariiformis* sister to a larger cluster of OTUs predominantly referable to subsp. *bulbilifer*. Both recovered clusters contained OTUs referable to either subspecies. The complete linkage analysis recovered three clusters within the *bulbilifer/ficariiformis* division—one of OTUs predominantly referable to *ficariiformis*, nested within two composed predominantly of OTUs referable to subsp. *bulbilifer*. Single linkage similarly recovered a cluster of OTUs referable to subsp. *ficariiformis* nested within OTUs referable to subsp. *bulbilifer*. In the *calthifolius/chrysocephalus/ficaria* division, both average and complete linkage analyses recovered a cluster of OTUs predominantly referable to subsp. *ficaria* nested within clusters of OTUs predominantly referable to subsp. *calthifolius*. OTUs referable to subsp. *ficaria* did not emerge in a distinct cluster in the single linkage analysis, but rather were interspersed throughout those referable to subsp. *calthifolius*. OTUs referable to subsp. *chrysocephalus* emerged interspersed in grades of OTUs referable to subsp. *calthifolius* and subsp. *ficaria* in a sister position to the rest of the division in all three analyses.

PCoA and PCA

Consistent with cluster analysis results, two non-overlapping clusters of OTUs were recovered in the PCoA corresponding to (1) the bulbil bearing taxa: subsp. *bulbilifer* and subsp. *ficariiformis* sensu Sell (1994) and (2) the non-bulbil bearing taxa: subsp. *calthifolius*, subsp. *chrysocephalus*, and subsp. *ficaria* sensu Sell (1994; Fig. 3A). Within both of these clusters, cohesiveness was exhibited by each group of OTUs referable to one of the five subspecies sensu Sell (1994), although each group overlapped with another to some degree.

Consistent with expectations for infraspecific entities, distinct but overlapping clusters of OTUs were

TABLE 1. Non-transformed means and standard deviations (s.d.) of seven characters among the five putative subspecies of *Ranunculus ficaria* (format: mean (s.d.; N)). F statistic and p-values for one-way ANOVAs of log_{10} transformed data provided in ultimate two columns. Within a row, means with different superscripts differ significantly (p<0.05) when component values log_{10} transformed and analyzed using post-hoc tests (Tukey's HSD).

	bulbilifer	calthifolius	chrysocephalus	ficaria	ficariiformis	F	p-value
Leaf length (cm)	2.18 ^{a,d} (0.41; 82)	1.93 ^{a} (0.46; 96)	3.54 ^{b} (1.03; 6)	2.42 ^{d} (0.39; 37)	2.97 ^{c} (0.49; 12)	9.79	<0.001
Leaf width (cm)	2.83 ^{a} (0.53; 82)	2.38 ^{b} (0.54; 97)	4.33 ^{d} (1.15; 6)	2.95 ^{a,d} (0.48; 37)	3.80 ^{c,d} (0.53; 12)	10.56	<0.001
Petiole length (cm)	10.41 ^{a} (2.79; 82)	6.55 ^{b} (1.62; 97)	16.21 ^{c} (1.44; 6)	12.19 ^{a,c,d} (2.16; 37)	14.75 ^{c,d} (3.85; 12)	28.44	<0.001
Petal length (mm)	10.23 ^{a} (1.67; 75)	11.11 ^{a} (1.91; 97)	17.43 ^{b,d} (3.79; 6)	12.11 ^{a,c} (1.95; 37)	13.91 ^{c,d} (1.95; 11)	16.89	<0.001
Petal width (mm)	3.57 ^{a} (0.91; 75)	4.53 ^{b} (1.05; 97)	7.38 ^{c} (0.45; 6)	4.83 ^{b,d} (1.09; 37)	5.75 ^{d} (1.14; 11)	23.62	<0.001
Achene length (mm)	2.95 ^{a} (0.65; 53)	3.04 ^{a} (0.65; 28)	4.33 ^{b,c} (0.69; 5)	3.27 ^{a,c} (0.41; 6)	3.50 ^{a,c} (0.90; 7)	5.30	<0.001
Achene width (mm)	4.35 ^{a} (0.90; 53)	4.26 ^{a} (1.00; 28)	6.22 ^{b,c} (0.77; 5)	4.58 ^{a,c} (1.12; 6)	4.70 ^{a,c} (1.31; 7)	3.83	<0.01

exhibited in the PCA comprising all OTUs when symbol coded for a priori subspecies assignments following the infraspecific concepts of Sell (1994) (Fig. 3B). Among these, OTUs referable to subsp. *bulbilifer* exhibited the most cohesive and least diffuse cluster. In this analysis, 81% of the variation is explained by the first two axes. PC1 is positively correlated most strongly with petiole length, leaf width, and leaf length, whereas PC2 is positively correlated most strongly with petal width and petal length (Table 2).

A separate analysis of only OTUs referred to the two diploid taxa subsp. *calthifolius* and *ficaria*, resulted in two very well-defined clusters with minor overlap (Fig. 3C; Table 3). In this analysis, 73 % of variation is explained by the first two axes. PC1 is positively correlated most strongly with petiole length, leaf length, and leaf width, whereas PC2 is positively correlated most strongly with petal width and petal length (Table 3). An analysis of only OTUs referred to the tetraploid taxa—subsp. *bulbilifer*, *chrysocephalus*, and *ficariiformis*—showed evident clustering, but with greater overlap among the three a priori defined subspecies (Fig. 3D; Table 4). In this analysis, 76% of the variation is explained by the first two axes. OTUs defined a priori as subsp. *ficariiformis* occupied a central coordinate space in the tetraploid analysis, flanked along the primary axis by subsp. *chrysocephalus* to the left and *bulbilifer* to the right. PC1 is negatively correlated most strongly with petal width, leaf length, and leaf width, whereas PC2 is positively correlated most strongly with petiole length (Table 4).

Among the five subspecies of *R. ficaria* recognized by Sell (1994), only the tetraploid subsp. *bulbilifer* and *ficariiformis* are known to produce bulbils. A separate analyses of only OTUs with bulbils, showed two rather cohesive clusters with limited overlap corresponding to a priori assignment to these two subspecies sensu Sell (1994; Fig. 3E; Table 5). In this analysis, 69% of the variation is explained by the first two axes. PC1 is negatively correlated most strongly with petal width, whereas PC2 is positively correlated most strongly with petal width and petal length (Table 5). A separate analysis of OTUs without bulbils (Fig. 3f; Table 6), showed three rather cohesive clusters with limited overlap, corresponding to a priori assigned subspecies. In this analysis, 79% of the variation is explained by the first two axes. PC1 is positively correlated most strongly with petiole length, leaf width, and leaf length, whereas PC2 is positively correlated most strongly with petal width and petal length (Table 6).

Classification tree

Classification tree analysis showed that 95% or greater of the OTUs we referred to subsp. *calthifolius*, subsp. *ficaria*, and subsp. *bulbilifer* using Sell (1994) could be placed into corresponding homogeneous groups (Fig. 4).

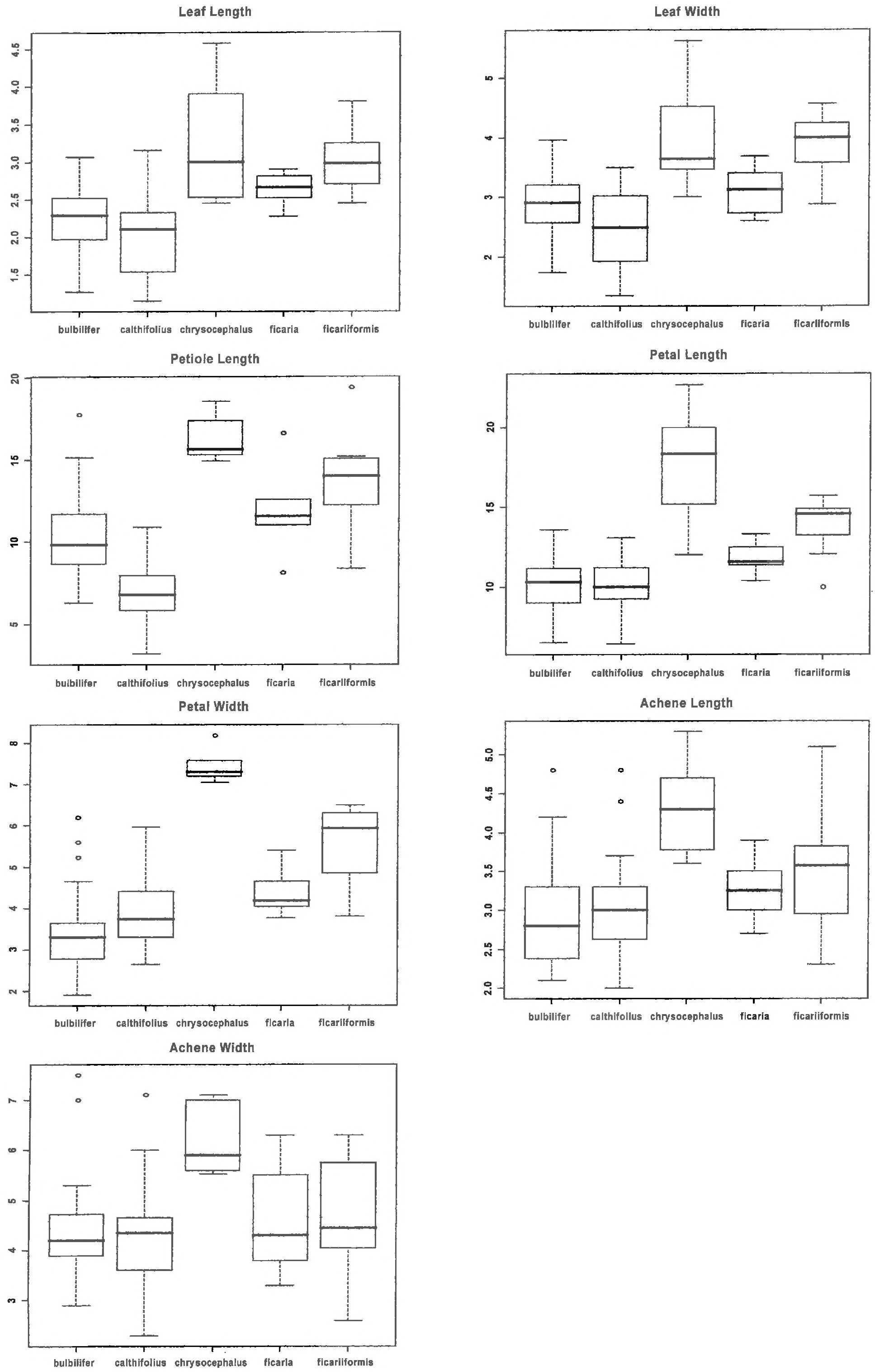

Fig. 1. Boxplots showing distribution of non-transformed measurements for seven quantitative characters in *Ranunculus ficaria* in the United States. Y-axis units are mm, except for leaf characters, which are in cm.

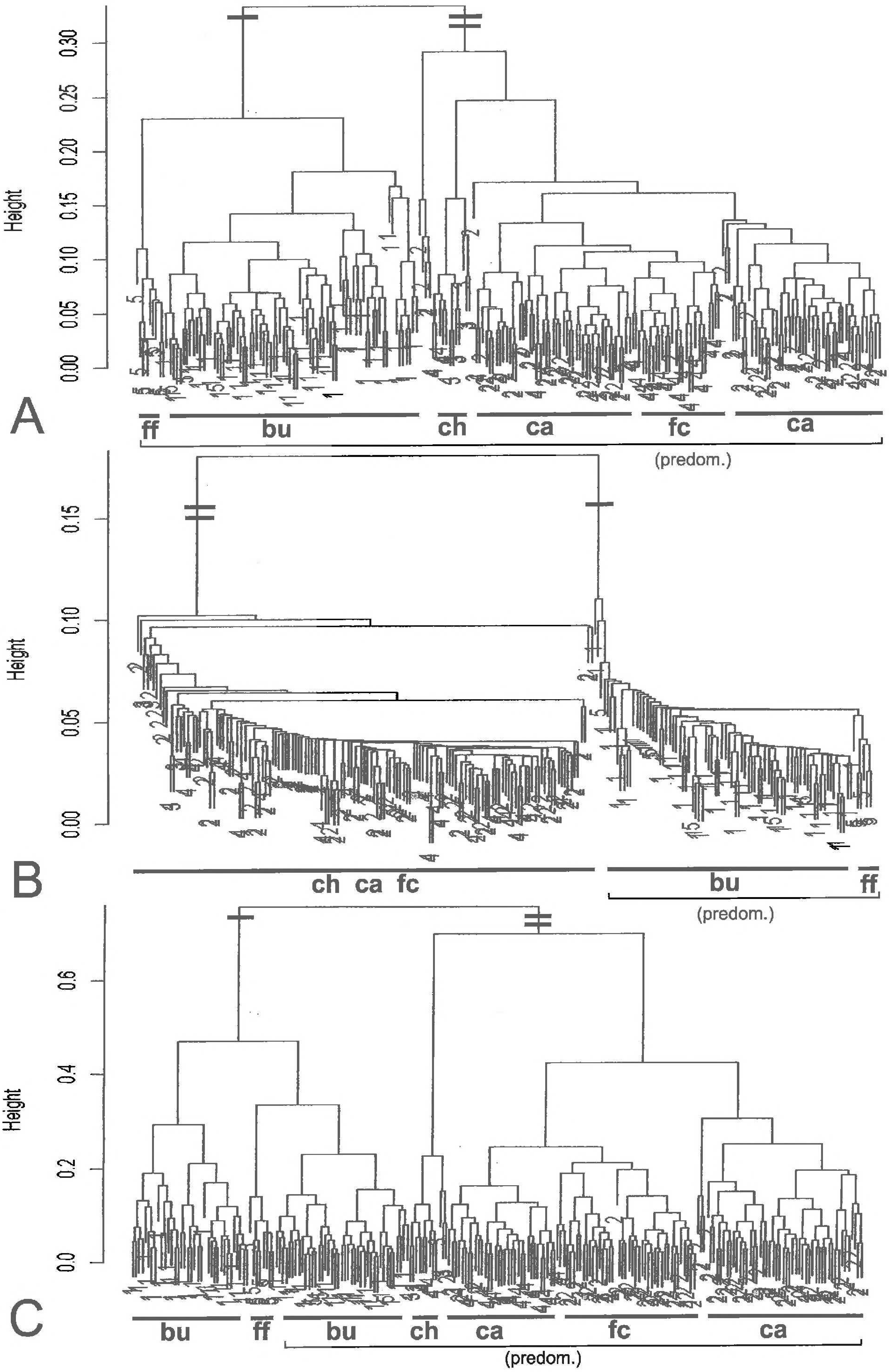

Fig. 2. Cluster analyses of OTUs of *Ranunculus ficaria* in the United States: A, Average linkage (UPGMA); B, Single linkage; C, Complete linkage. Single bar indicates bulbiliferous OTUs. Double bar indicates non-bulbiliferous OTUs. In cases where OTUs sorted incompletely, the predominant taxon in a delineated cluster is indicated followed by "predom." bu = subsp. *bulbilifer* (1); ca = subsp. *calthifolius* (2); ch = subsp. *chrysocephalus* (3); fc = subsp. *ficaria* (4); ff = subsp. *ficariiformis* (5).

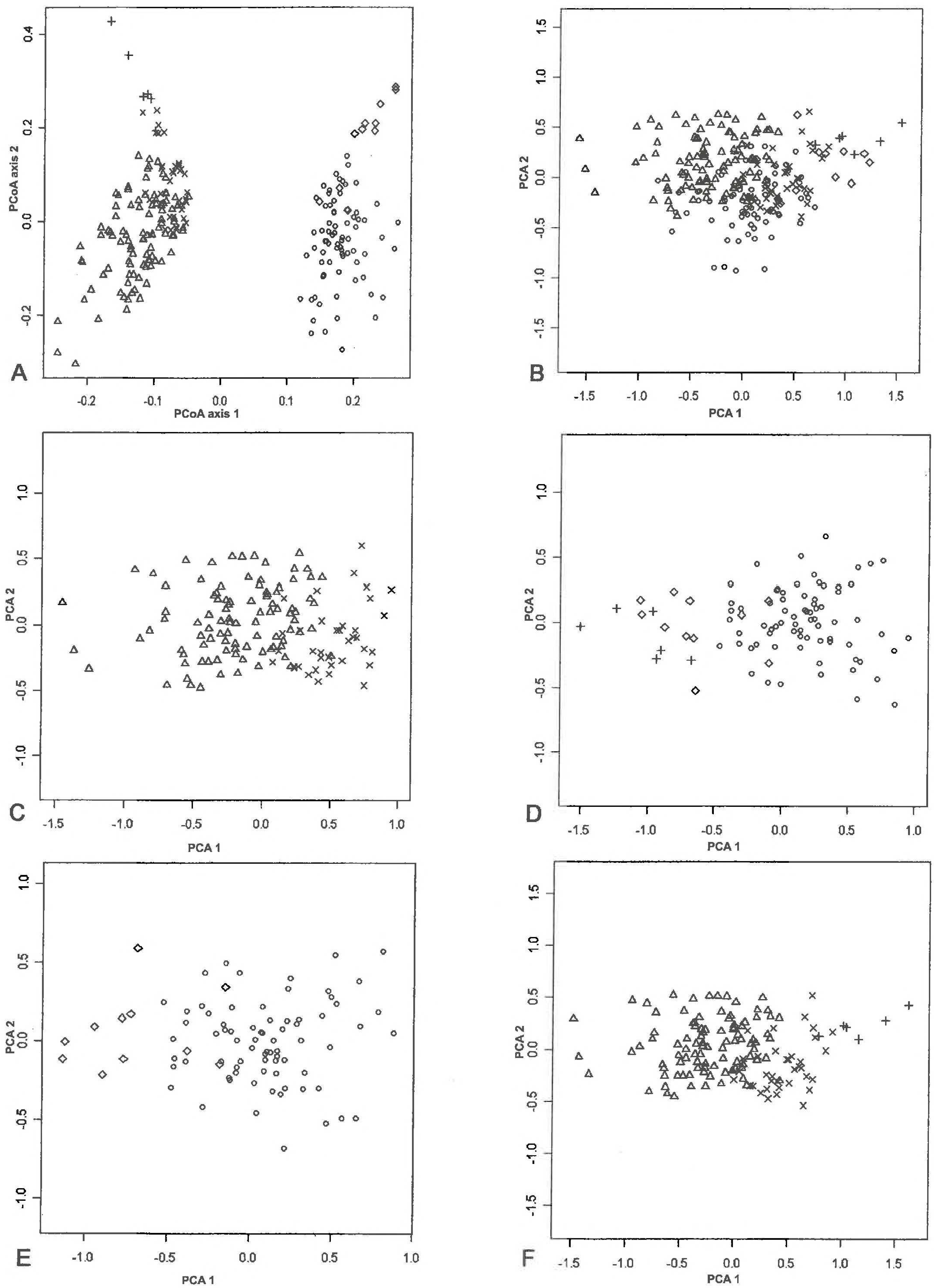

Fig. 3. Ordination results for Principal Coordinates Analysis (A: all OTUs) and Principal Components Analyses (B–F); B: all OTUs; C: putative diploid OTUs; D: putative tetraploid OTUs; E: bulbiliferous OTUs; F: non-bulbiliferous OTUs. ○ = subsp. ***bulbilifer***; Δ = subsp. ***calthifolius***; + = subsp. ***chrysocephalus***; x = subsp. ***ficaria***; ◊ = subsp. ***ficariiformis***

Table 2. Character loadings for the first three principal components (PC) in the combined PCA of all OTUs.

	PC1	PC2	PC3
Leaf length	0.4447150	0.06007699	-0.5807531
Leaf width	0.4659988	0.01441147	-0.4945180
Petiole length	0.6942403	-0.45147042	0.5562742
Petal length	0.2116039	0.44013398	0.1484652
Petal width	0.2415118	0.77371804	0.2944399

Table 3. Character loadings for the first three principal components (PC) in the PCA of the putative diploid OTUs.

	PC1	PC2	PC3
Leaf length	0.4393161	0.1933816	-0.5527152
Leaf width	0.4473662	0.1682932	-0.4790156
Petiole length	0.7371736	-0.4948622	0.4585902
Petal length	0.1785614	0.4539478	0.2102479
Petal width	0.1776393	0.6952149	0.4588473

Table 4. Character loadings for the first three principal components (PC) in the PCA of the putative tetraploid OTUs.

	PC1	PC2	PC3
Leaf length	-0.4145495	0.2908487	-0.5513932
Leaf width	-0.4139542	0.2858958	-0.4349598
Petiole length	-0.4282971	0.5777908	0.6924294
Petal length	-0.3705260	-0.3867720	0.1113257
Petal width	-0.5797091	-0.5918072	0.1221624

Table 5. Character loadings for the first three principal components (PC) in the PCA of the bulbiliferous OTUs.

	PC1	PC2	PC3
Leaf length	-0.4166727	-0.2045966	0.5826665
Leaf width	-0.4395120	-0.2045104	0.4630089
Petiole length	-0.4585246	-0.6345035	-0.6193181
Petal length	-0.3303427	0.3747980	-0.1413127
Petal width	-0.5602160	0.6109399	-0.2063936

Table 6. Character loadings for the first three principal components (PC) in the PCA of the non-bulbiliferous OTUs.

	PC1	PC2	PC3
Leaf length	0.4474063	0.1388268	-0.5661383
Leaf width	0.4522951	0.1139208	-0.4887299
Petiole length	0.6954902	-0.5497825	0.4608629
Petal length	0.2280402	0.4510623	0.2216459
Petal width	0.2440242	0.6797288	0.4232127

Seventy-five percent (N=6) and 87.5% (N=7) of OTUs we referred respectively to subsp. *chrysocephalus* and subsp. *ficariiformis* using Sell (1994) could be placed into corresponding homogeneous groups. A quarter of the OTUs (N=2) assigned to subsp. *chrysocephalus* in the analysis were specimens we referred to subsp. *ficaria* using Sell (1994). The presence of bulbils separated the *calthifolius/chrysocephalus/ficaria* group from

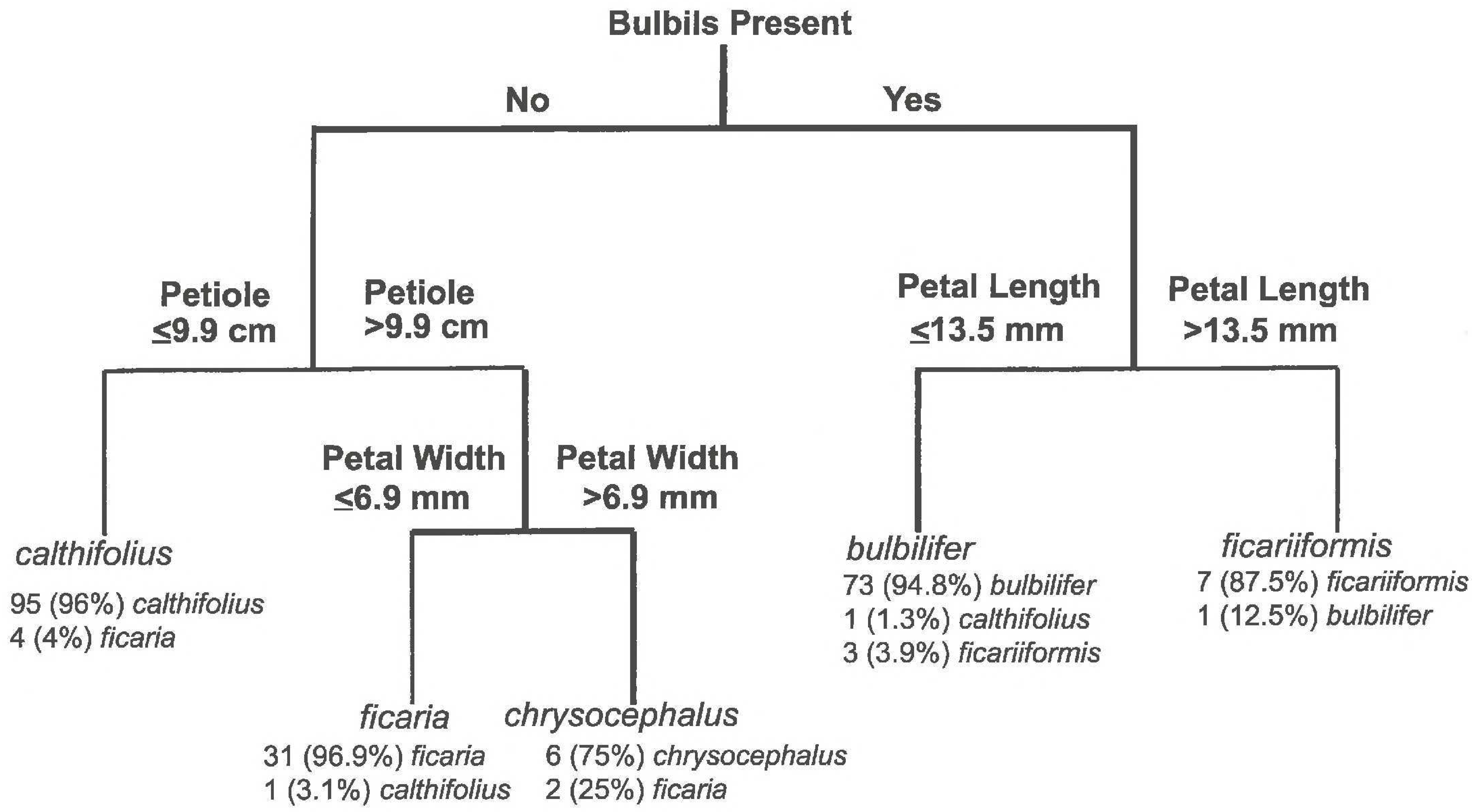

Fig. 4. Classification tree analysis. Numbers given at node terminals indicate our *a priori* classifications sensu Sell (1994). For example, the model assigned ninety-nine OTUs to the *calthifolius* node terminal. Ninety-five OTUs (96%) from this node were assigned to *calthifolius* by us and four (4%) were assigned to *ficaria* by us.

the *bulbilifer/ficariiformis* group. Within the former, petiole length discriminated best between OTUs referable to subsp. *calthifolius* and those referable to subsp. *ficaria* and subsp. *chrysocephalus*. Petal width discriminated best between subsp. *ficaria* and subsp. *chrysocephalus*. Petal length discriminated best between subsp. *bulbilifer* and subsp. *ficariiformis*. The shape of bulbils was not scored for the classification tree analysis as we were interested in seeing what additional vegetative character distinguished these putative taxa.

DISCUSSION

Subspecies recognition.—The combined results indicate the presence of five entities that can be reasonably referred to the subspecies accepted by Sell (1994). If one accepts subspecies as incompletely diverged lineages, one would expect a limited amount of overlap of OTUs as seen in our PCoA and PCA results, as well as incompletely sorted OTUs as seen in our cluster and classification tree analyses (Rosen et al. 2007). The ANOVA results are also informative on this issue, particularly because the assignment of each OTU to a putative subspecies was based exclusively on the key by Sell (1994; see above). In this key, quantitative measurements were used only to distinguish two groups of subspecies (i.e., *chrysocephalus/ficariiformis* and *bulbilifer/calthifolius/ficaria*). *Qualitative* characters are used in Sell's (1994) key to distinguish individual subspecies within these two groups. Thus, contributing evidence of the morphological cohesiveness of the subspecies concept of Sell (1994) is the extent to which differences in quantitative characters are found between all subspecies pairs. Of course, had we found that our OTU groups assigned to the subspecies sensu Sell (1994) did not differ significantly in quantitative characters, it would not necessarily have challenged Sell's concepts, as the taxa may truly differ only in qualitative characters. However, the finding that the OTU groups corresponding to the subspecies sensu Sell (1994) do in fact differ in various combinations of the quantitative characters we examined provides some additional evidence of distinctness. Although the groups differed primarily in the means of quantitative characters and showed overlap in maximum dimensions, if one accepts a subspecies as an incompletely diverged lineage, overlap in character states cannot be unexpected. In addition, distinct means in quantitative characters could be viewed as a reflection of partial isolation and potentially emerging distinct evolutionary trajectories, possibly leading to speciation. In recognizing that our analysis is limited to plants introduced to the United States, our results could be biased if our data sets largely contained "non-controversial" individuals (e.g., individuals from subspecific centers of distribution in Europe, rather than regions of overlap). There is no way to know this, except through a broader study. However, we did not explicitly seek to test the subspecies concept sensu Sell (1994), but rather whether plants introduced to the United States could be reasonably referred to that concept—which we believe they can. The recognition of subspecies of *R. ficaria* has obvious practical consequences in weed management, as not all subspecies may behave in the same manner.

Summary of taxon distribution, habitat, and rate of spread.—Based on our current understanding, the subspecies of *R. ficaria* exhibit overlapping distributions in the United States (Fig. 5). Subspecies *calthifolius* occurs in eighteen states and the District of Columbia. It was apparently first collected in the United States in 1867 (Pennsylvania: *Burke s.n.*, PH). Collections of this subspecies account for 35.5% of specimens examined. Subspecies *bulbilifer* currently occurs in sixteen states and the District of Columbia. It was apparently first collected in the United States in 1891 (New York: *Hollick s.n*, LGO) and accounts for 31.5% of the specimens examined. Subspecies *ficaria* occurs in ten states and the District of Columbia. It was apparently first collected in the United States in 1876 (New York: *Schrenck s.n.*, LGO) and accounts for 15% of the collections examined. Subspecies *ficariiformis* currently occurs in Missouri, North Carolina, New York, Ohio, Pennsylvania, and Texas. It was apparently first collected in the United States in 1910 (Pennsylvania: *St. John 111*, GH) and accounts for only 8% of specimens examined. Subspecies *chrysocephalus* currently is known only from Maryland, New York, Oregon, and Washington. It was apparently first collected in the United States in 1975 (Oregon: *Hatch s.n.*, NY, OSC). The narrower range documented for subsp. *chrysocephalus* vis-à-vis the other subspecies is attributed to the recency of introduction—collections of this subspecies in the United States are unknown prior to 1975. The disjunct populations in the east and west likely resulted from secondary introductions through the horticultural trade.

All subspecies of *Ranunculus ficaria* are best adapted to moist sites (Taylor & Markham 1978). All perform well in irrigated landscapes, such as lawns and horticultural plantings, but occur in a variety of habitats from moist woods to roadsides and lawns (Fig. 6). The majority of collections of all subspecies (45.9%) were made adjacent to a water source such as a river, stream, or pond. An additional 15.5% came from other moist areas. Collections from lawns and horticultural plantings were equal at 8.13% each. A few specimens have been collected in other habitats such as disturbed sites, lowlands, and fields. Twenty-three percent of specimens of subsp. *ficariiformis* were collected from dry woods suggesting that this subspecies may tolerate more xeric environments than the other four. Habitat distributions of subsp. *chrysocephalus* and subsp. *ficariiformis* likely represent only a limited percent of the habitat range of these taxa due to the limited number of collections they are based upon (N = 6 and N = 13, respectively).

Vegetative spread occurs through tuberous roots, although subsp. *bulbilifer* and subsp. *ficariiformis* also produce axillary bulbils for reproduction (Taylor & Markham 1978; Sell 1994). All subspecies except subsp. *bulbilifer* produce viable seed which frequently fall adjacent to parent plants (Marsden-Jones 1937). Dispersal over long distances likely occurs anthropogenically. The subspecies multiply easily along riverbanks, forming dense mats where there is seasonal flooding (Taylor & Markham 1978). Short distance dispersal is effected by seasonal flood waters which may transport tubers or bulbils downstream. This dispersal pattern was confirmed by primary observation on subsp. *ficariiformis* in Wake Co., North Carolina, in the spring of 2006. The subspecies was distributed along a drainage ditch, through a culvert under the road, and into a local waterway where it colonized banks downstream from the source. Persistence in the landscape is exacerbated by continued use in the nursery trade as a garden plant. Plants may slowly escape from cultivation and spread when tuberous roots, bulbils, or small plants are discarded in yard waste.

Figure 7 shows the relative rate of spread of each subspecies by the number of counties in which it was collected by decade. It does not appear that any single subspecies of *R. ficaria* is more invasive than another

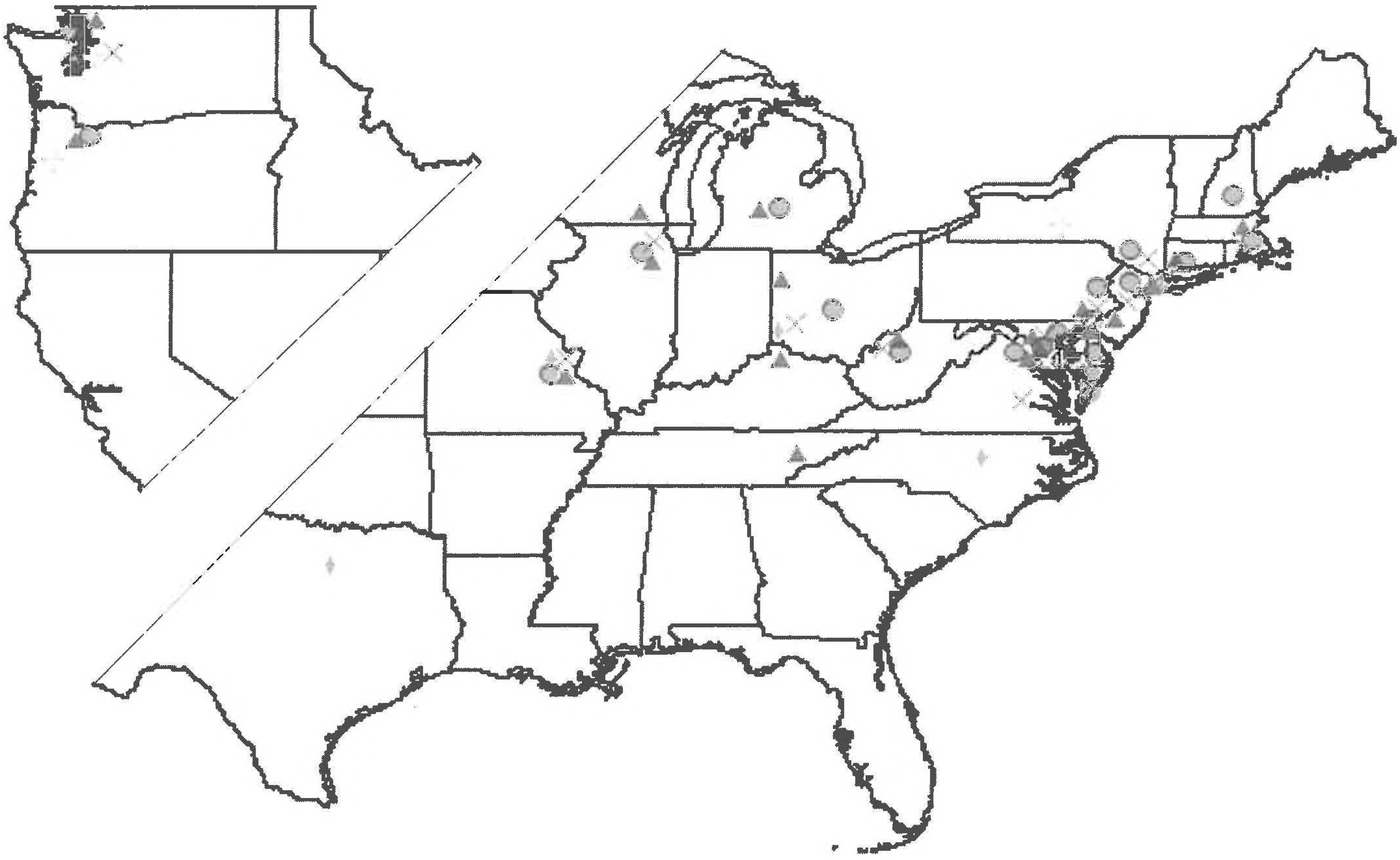

FIG. 5. Distribution of *Ranunculus ficaria* and its five subspecies in the United States as of 2008. ● = subsp. *bulbilifer*; ▲ = subsp. *calthifolius*; + = subsp. *chrysocephalus*; ★ = subsp. *ficaria*; ♦ = subsp. *ficariiformis*.

in the United States. There is no significant difference among the expansion slopes of the five subspecies during the first forty years after each introduction (p=0.0769), suggesting that each subspecies behaves similarly, at least in the early phases of expansion. It may be expected, therefore, that more recently introduced taxa, such as subsp. *chrysocephalus*, subsp. *ficaria*, and subsp. *ficariiformis*, will follow a similar pattern to that of the earlier introductions, subsp. *bulbilifer* and subsp. *calthifolius*, and extend their ranges at similar exponential growth rates in the next hundred years.

All subspecies of *R. ficaria* should be expected to persist where introduced throughout most of Canada, New England to Iowa, possibly northern California, and as far south as Texas. We have not seen collections from California, Iowa, Indiana, Maine, Rhode Island, or Vermont, although expect that subspecies will persist there, as well. The Midwestern states of Arizona, Colorado, Idaho, Kansas, North Dakota, Nebraska, Nevada, Oklahoma, South Dakota, Utah, and Wyoming are likely too arid for widespread naturalization. However, subspecies may establish locally in irrigated areas or wetlands following introduction.

APPENDIX A

List of exsiccatae of *Ranunculus ficaria* in the United States. Arranged alphabetically by subspecies. * = handwriting difficult to decipher; ♣ = double flowers.

Ranunculus ficaria L. subsp. **bulbilifer** Lambinon

U.S.A. CONNECTICUT. New Haven Co.: 12 May 1992, *Mehrhoff 15469* (CONN, Y); 4 Jun 1997, *Morehead III 3561* (CONN). **DELAWARE. New Castle Co.:** 24 Apr 2004, *Clancy 5905* (DOV); Apr 1978, *Lindtner 109* (DOV); *Schuyler 7210* (PH). **DISTRICT OF COLUMBIA:** 27 Apr 1993, *Redman 6651* (BALT). **ILLINOIS. Cook Co.:** 6 May 1987, *Evert 11832* (MOR); 6 May 1987, *Evert 11833* (MOR); 17 May 1989, *Evert 16279* (MOR, NA); 8 May 1997, *Hickman 514* (MOR); 26 May 1978, *Kamin 945-3000* (MOR); 2 May 1982, *Lace s.n.* (MOR); 5 May 1998, *Masi, Epting, & Kossovich 817* (ILLS); 1 May 1960, *Venrick 122* (MO). **Dupage Co.:** 27 Apr 2003, *Kobal FPD03-02* (MOR); 5 May 1995, *Lampa 95-03* (MOR). **Lake Co.:** 27 May 1998, *Fiest 24* (ILLS). **MARYLAND. Baltimore Co.:** 26 Apr 1975, *Beach 0107* (MARY); 17 Apr 1984, *Hill 13552* (BRIT, GH, MARY, MO, MSC, NY-2 sheets, POM); 22 Mar 1989, *Redman 6320* (BALT); 30 Apr 1993, *Redman 6492* (BALT); 14 Apr 1974, *Romeo 41* (MARY). **Howard Co.:** 30 Apr 1965, *Engh s.n.* (BKL, MARY).

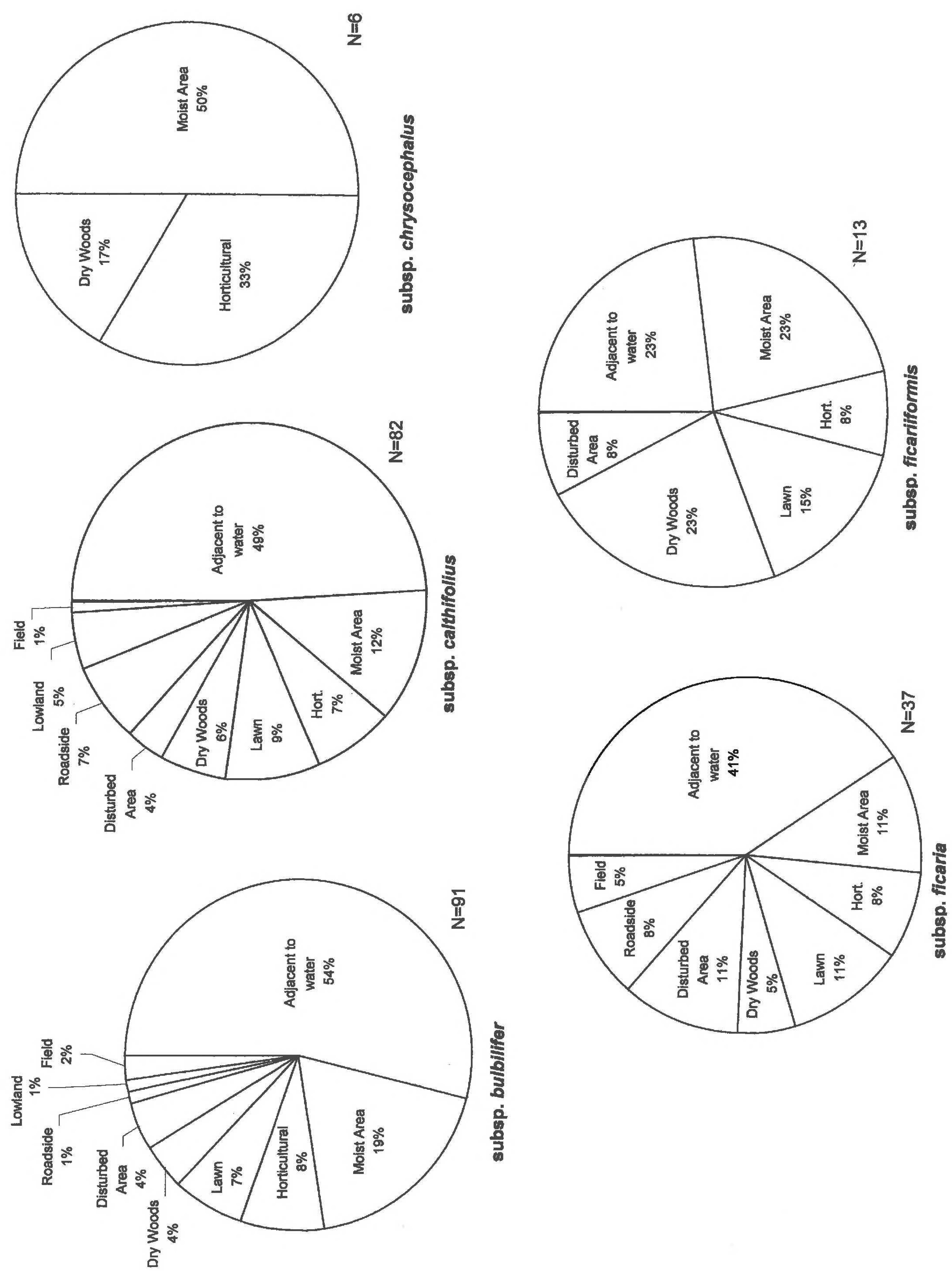

Fig. 6. Percentage of collections from various habitat for each of five subspecies of *Ranunculus ficaria* in the United States.

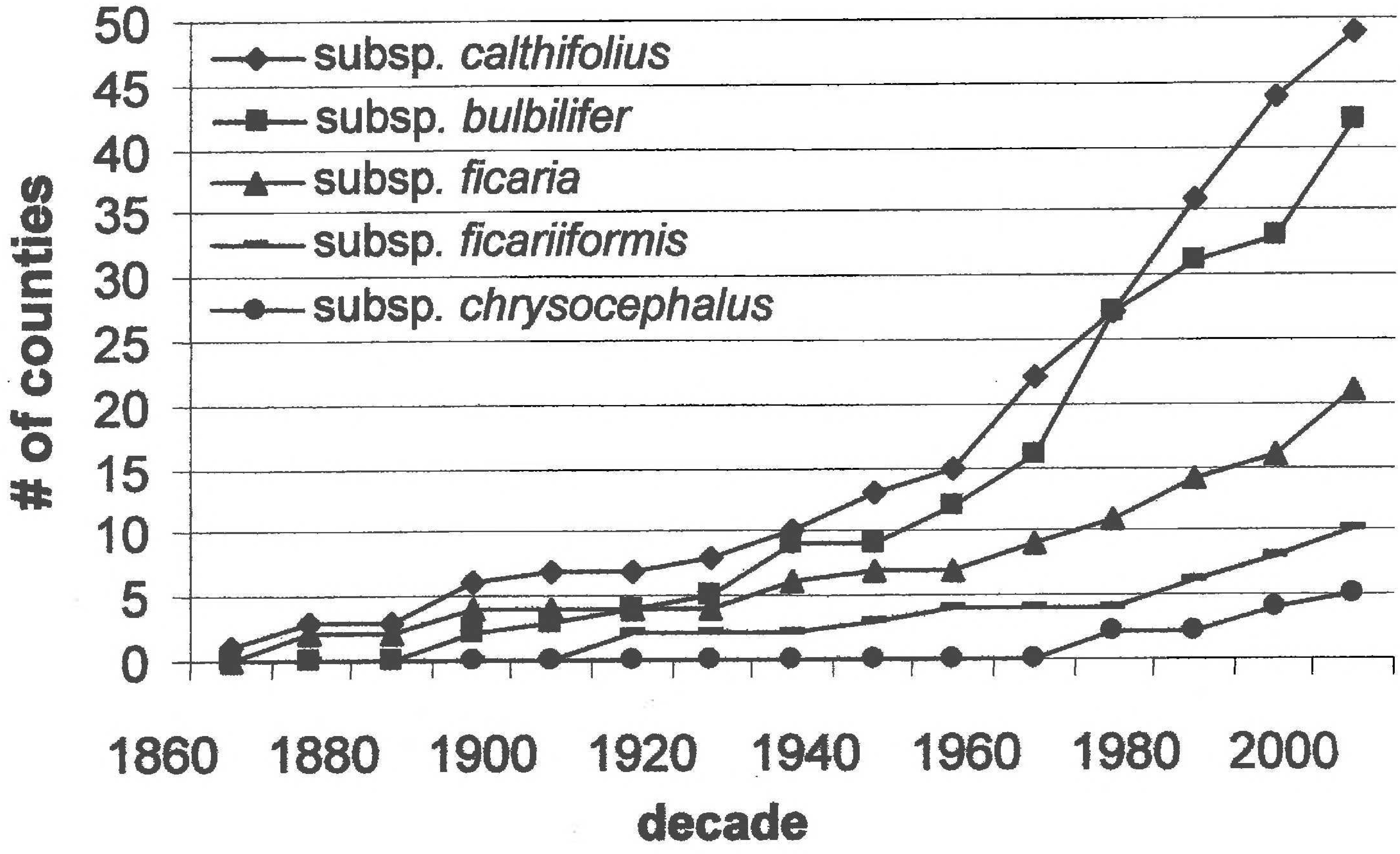

Fig. 7. Rate of spread of each subspecies of *Ranunculus ficaria* based on number of county records per decade. There is no significant difference among the expansion slopes of the five subspecies during the first forty years after each introduction (p = 0.0769).

Montgomery Co.: 25 Apr 1937, *Benedict Jr. 3679* (NA); 18 Apr 1976, *Morris 11* (MARY). **Prince Georges Co.:** 19 Apr 1974, *Bunn 37* (MARY). **MASSACHUSETTS. Suffolk Co.:** 24 May 2004, *Mehrhoff 21169* (CONN). **MICHIGAN. Clinton Co.:** 9 May 1982, *Gereau 966* (MICH, MSC). **Eaton Co.:** 22 May 1984, *Blouch s.n.* (MSC); 31 May 1984, *Blouch s.n.* (MSC). **Ionia Co.:** 21 May 2003, *Reznicek & Kogge 11470* (MICH). **MISSOURI. Saint Louis Co.:** 21 Apr 1985, *Brant 551* (MO); 26 Apr 1972, *Brown s.n.* (MO); 12 Apr 1992, *Ochs 19* (MO). **NEW HAMPSHIRE. Hillsborough Co.:** 13 May 1962, *Stiff s.n.* (NHA). **NEW JERSEY. Essex Co.:** 25 Apr 2003, *Glenn 8181* (BKL). **Hunterdon Co.:** 26 Apr 2001, *Glenn 5425* (BKL). **Mercer Co.:** 22 Apr 2002, *Glenn 8988* (CONN, BKL). **Passaic Co.:** 17 May 1939, *Clausen s.n.* (BH); 6 May 1941, *Langmuir & Lawrence s.n.* (BH). **Somerset Co.:** 23 Apr 2003, *Glenn 8149* (BKL). **NEW YORK. Bronx Co.:** 4 May 1964, *Bennett s.n.* (NY); 28 Apr 1939, *Swift R432/37* (NY); 4 May 1996, *Walker 1868* (NY); 19 Apr 1989, *Yost 355* (DOV). **Cayuga Co.:** 23 May 1932, *Hazard 17883* (CU). **Nassau Co.:** 27 Apr 2001, *Steward 416* (BKL). **Richmond Co.:** 3 May 1937, *Guiler s.n.* (CU); 19 Apr 1891, *Hollick s.n.* (LGO). **Tompkins Co.:** 10 May 1935, *Burnham 18847* (CU); 22 May 1935, *Clausen s.n.* (BH); 22 May 1917, *Gershoy 8104* (CU). **OHIO. Franklin Co.:** 17 Apr 1977, *Carr 68* (OS); 27 Apr 1980, *Carr 2645* (OS); 28 Apr 1987, *Lammers 6084* (OS); 16 Apr 1992, *Lowden 4908* (OS). **Hamilton Co.:** 21 Apr 1982, *Cusick 21469* (OS); 12 Apr 1989, *Cusick 27953* (OS). **Montgomery Co.:** 6 May 1978, *Cusick 18074* (KE, OS); 23 Apr 1998, *McCormac 6484* (MICH). **OREGON. Multnomah Co.:** 26 Apr 1962, *Smith s.n.* (OSC). **PENNSYLVANIA. Berks Co.:** 5 May 1972, *Brumbach 7910* (BH, NA-2 sheets, NY). **Blair Co.:** 1 May 1987, *Kunsman 8564* (PH). **Bucks Co.:** 3 May 1959, *Forman s.n.* (PH); 18 Apr 1998, *Mehrhoff 20039* (CONN). **Chester Co.:** 18 Apr 1959, *Webb & Wherry s.n.* (PH). **Delaware Co.:** 6 Apr 1937, *Blaser s.n.* (CU); 7 May 1944, *Carter 5084* (DOV); Apr 1908, *Painter s.n.* (MO, NA); 16 Apr 1942, *Wheeler 5600* (POM). **LeHigh Co.:** 26 Apr 1959, *Schaeffer Jr. 58388* (PH). **Montgomery Co.:** 22 Apr 1922, *Dreisbach 868* (F, MICH); 24 Apr 1954, *Wherry s.n.* (PH). **Philadelphia Co.:** 26 Apr 1911, *Eckfelds s.n.* (PH-2 sheets); 1 May 1912, *Fackenthall s.n.* (NA); 20 Apr 1911, *St. John s.n.* (GH); 12 Apr 1908, *Van Pelt s.n.* (MICH, PH). **VIRGINIA. Fairfax Co.:** 13 Apr 1976, *Bradley & Frederickson 9954* (WVA). **WASHINGTON. King Co.:** 17 Mar 2002, *Zika & Jacobson 16885* (WTU). **WEST VIRGINIA. Ritchie Co.:** 15 May 1970, *Elliott s.n.* (WVA). **Wood Co.:** 8 Apr 2003, *Grafton s.n.* (WVA).

Ranunculus ficaria L. subsp. **calthifolius** (Reichenb.) Arcangeli

U.S.A. CONNECTICUT. Fairfield Co.: 16 Apr 1985, *Mehrhoff 11192* (CONN). **New Haven Co.:** 20 Apr 2001, *Murray 01-001* (CONN). **Tolland Co.:** 29 Apr 2000, *Mehrhoff 20744* (CONN). **DELAWARE. New Castle Co.:** 4 Apr 1985, *Meyer & Mazzeo 20693* (NA). **DISTRICT OF COLUMBIA:** 20 Mar 1983, *Flemming s.n.* (MARY); 14 Apr 1983, *Fleming s.n.* (NA); 3 Apr 1986, *Fleming 4* (NA); 13 Apr 1899, *Maxon* 72* (NA); ♣ 1874, *McCarthy s.n.* (NA); 26 Apr 1884, *McCarthy s.n.* (NA); 18 Apr 1897, *Topping s.n.* (NA). **ILLINOIS. Cook Co.:** 2 May 1996, *Antonio & Masi 7577* (ILLS); 24 Apr 1966, *Argent M. D. s.n.* (ILLS). **Lake Co.:** 24 Apr 1988, *Snydacker*

561 (F). **KENTUCKY. Campbell Co.:** 12 Apr 1981, *Buddell II 108* (NY). **MARYLAND. Anne Arundel Co.:** 4 Apr 1991, *Longbottom 1460* (MARY); 15 Apr 1993, *Longbottom 3473* (MARY). **Baltimore Co.:** 23 Apr 1971, *Chanoski 043* (BALT); 3 Apr 1980, *Critikos 9* (BALT); 4 Apr 1977, *Lears s.n.* (MARY); 13 Apr 1974, *Ness 33* (MARY); 1 May 1984, *Redman 4029* (BALT). **Caroll Co.:** 13 Apr 1963, *Burroughs 31* (MARY). **Howard Co.:** 17 Apr 1965, *Stolze 386* (F-2 sheets). **Montgomery Co.:** 25 Jan 1950, *Cross s.n.* (NA); 27 Mar 1976, *Mora 22* (MARY); 1 Apr 1971, *Sappington s.n.* (MARY); 6 Apr 1975, *Schlossberg 0002* (MARY); 19 Mar 1983, *Zastrow 1* (OSC). **Prince Georges Co.:** 9 Apr 1987, *Bowman 377* (MARY); 13 Apr 1970, *Thompson Jr. s.n.* (MARY). **MASSACHUSETTS. Middlesex Co.:** 8 May 1982, *Wood 4690* (MT). **MICHIGAN. Ionia Co.:** 23 Apr 1989, *Penskar 1085* (MICH); 24 Apr 1989, *Penskar 1086* (MICH). **MISSOURI. Saint Louis Co.:** 4 Apr 1989, *Yatskievych, Yatskievych, Denison 89-05* (MO); 1 Apr 1999, *Yatskievych, Yatskievych, Harris, Harris, & Summers 99-04* (MO). **NEW JERSEY. Burlington Co.:** 16 Apr 1932, *Stokes M.D. s.n.* (PH). **Camden Co.:** 23 Apr 1898, *Saunders s.n.* (PH). **Middlesex Co.:** 12 Apr 2003, *Martine & Skogen 354* (CONN). **Somerset Co.:** 7 Apr 2000, *Glenn 4065* (BKL). **Union Co.:** 3 Apr 2002, *Glenn & Steward 6197* (BKL). **NEW YORK. Bronx Co.:** 11 Apr 1976, *Delendick s.n.* (BKL). **Dutchess Co.:** May 1941, *Van Melle s.n.* (BH). **Queens Co.:** Apr 1876, *Schrenk s.n.* (LGO); 1876, *Schrenk s.n.* (LGO); 25 Apr 1877, *Schrenk s.n.* (BKL, MO); Apr 1877, *Schrenk s.n.* (PH); 11 Apr 1878, *Schrenk s.n.* (MICH); Apr 1878, *Schrenk s.n.* (BKL, F-2 sheets, HNH, NA, NY-2 sheets, POM); Apr 1878, *Schrenk s.n.* (LGO-2 sheets, NA); May 1882, *Bisky s.n.* (BKL). **Richmond Co.:** 13 Apr 1898, *Coheu* s.n.* (BKL-2 sheets). **Tompkins Co.:** 7 May 1937, *Anderson s.n.* (MASS); ♣19 Apr 1959, *Dress 5984* (BH); 1 May 1996, *Dress 19996* (BH). **OHIO. Clark Co.:** 31 Mar 1992, *Cusick 30056* (OS). **Clermont Co.:** 2 Apr 1996, *Cusick 32886* (MO, OS). **Clinton Co.:** 3 Apr 1989, *Cusick 27946* (OS). **Franklin Co.:** 5 Apr 1986, *Cooperband 5* (OS); 29 Mar 1987, *Cusick 26207* (NY). **Greene Co.:** 31 Mar 1992, *Cusick 30054* (OS). **Lake Co.:** 2 Jun 1901, *Hacker s.n.* (OS). **Van Wert Co.:** 22 Apr 1946, *Brooks 1393* (OS); 28 Apr 1947, *Brooks s.n.* (OS). **OREGON. Multnomah Co.:** 27 Mar 1991, *Zika 11064* (OSC). **PENNSYLVANIA. Bucks Co.:** 18 Apr 1962, *Wherry s.n.* (PH). **Delaware Co.:** 13 Apr 1934, *Fogg Jr. 6316* (PH); 12 Apr 1938, *Fogg Jr. 14021* (GH); 7 Apr 1894, *MacElwee Jr. s.n.* (PH); 12 Apr 1920, *Meredith M.D. s.n.* (NY); 15 Apr 1942, *Schaeffer Jr. 16953* (PH-2 sheets); 19 Apr 1936, *Thompson Jr. 17* (PH); **Greene Co.:** 4 Apr 1953, *Buker s.n.* (PH). **Montgomery Co.:** ♣19 Apr 1963, *Fogg Jr. 22220* (A); 3 Apr 1921, *Long 23784* (PH); 20 Apr 1937, *Long 49720* (PH); 18 Feb 1954, *Long 77721* (PH); 1 May 1985, *Weaver s.n.* (PH). **Philadelphia Co.:** ♣1867, *Burke s.n.* (PH); ♣10 Apr 1954, *Fogg Jr. 21460* (PH-2 sheets); ♣18 Apr 1954, *Fogg Jr. 21474* (PH); 3 Apr 1933, *Hermann 3953* (NA); 18 Apr 1974, *Jers** (PH); ♣27 Apr 1924, *Lang 112* (GH); ♣1 May 1920, *Meredith M.D.* (NY); 30 Mar 1909, *St. John 110* (GH). **TENNESSEE. Knox Co.:** 17 Mar 1977, *DeSelm s.n.* (TENN); 25 Mar 1966, *Thomas s.n.* (BRIT); 31 Mar 1966, *Thomas & Rogers s.n.* (TENN). **VIRGINIA. Albemarle Co.:** 25 Mar 1997, *Stevens 25629* (VPI). **Chester Co.:** 3 Apr 1999, *Huber 1* (ILLS, OS). **Fairfax Co.:** 13 Apr 1947, *Sargent s.n.* (NCSC); 23 Mar 1974, *Sperling 36* (MARY). **WASHINGTON. Whatcom Co.:** 5 Apr 1968, *Sundquist 1550* (POM). **WEST VIRGINIA. Ritchie Co.:** 15 Apr 1963, *Stonestreet s.n.* (WVA). **WISCONSIN. Walworth Co.:** May 1970, *Larkin s.n.* (MOR).

Ranunculus ficaria L. subsp. **chrysocephalus** P.D. Sell
U.S.A. MARYLAND. Prince Georges Co.: 27 Mar 1977, *Wirick 05* (MARY). **NEW YORK. Tompkins Co.:** 18 May 1996, *Dress 15977* (CU). **OREGON. Benton Co.:** 31 Mar 1991, *Zika 11065* (OSC). **Lane Co.:** 22 Feb 1975, *Hatch s.n.* (NY, OSC). **WASHINGTON. King Co.:** 17 May 2002, *Zika & Jacobson 13733* (WTU).

Ranunculus ficaria L. subsp. **ficaria**
U.S.A. DISTRICT OF COLUMBIA: ♣ 29 Apr 1896, *Pollard 776* (NY); ♣29 Apr 1896, *Pollard 776* (MSC); ♣29 Apr 1896, *Pollard s.n.* (POM); ♣17 Apr 1898, *Pollard s.n.* (NY); ♣17 Apr 1898, *Steele s.n.* (MSC); ♣18 Apr 1897, *Steele s.n.* (GH); ♣ 18 Apr 1897, *Steele s.n.* (NA); ♣18 Apr 1897, *Steele s.n.* (NY). **ILLINOIS. Lake Co.:** 24 Apr 1988, *Snydacker 560* (MOR). **MARYLAND. Baltimore Co.:** 13 Apr 1980, *Caruso 22* (BALT); 5 Apr 1980, *King 10* (MARY). **Howard Co.:** 25 Mar 1989, *Redman 6321* (BALT). **Montgomery Co.:** 19 Apr 1975, *Hollenberg 8* (MARY); 19 Apr 1979, *Trumball 4* (BRIT); 13 Apr 1975, *Yinger 17* (MOR). **Prince Georges Co.:** 10 Apr 1977, *Dochtermann 29* (MARY); 16 Apr 1994, *Hedge 13* (MARY); 2 Apr 1982, *Kunowsky 10* (MARY); 4 Apr 1964, *Weigel Jr. s.n.* (MARY). **MASSACHUSETTS. Hampshire Co.:** 24 Apr 2002, *Mehrhoff 21611* (CONN, MASS). MISSOURI. Saint Louis Co.: 11 Apr 1990, *Christ s.n.* (MO); NEW JERSEY. Mercer Co.: 18 Apr 2001, *Glenn 5386* (BKL). Somerset Co.: 19 Apr 2001, *Glenn 5401* (BKL). NEW YORK. Cayuga Co.: 7 Jun 1935, *Petry 18846* (CU). Dutchess Co.: 1932, *Van Melle s.n.* (BH). Queens Co.: 22 Apr 1990, *Greller s.n.* (BKL); 1921, *Martin s.n.* (PH); Apr 1877, *Redfield 10896* (MO); Apr 1876, *Schrenk s.n.* (LGO). OHIO. Butler Co.: 2 Apr 1997, *Turner 31* (MU). PENNSYLVANIA. Chester Co.: ♣22 Apr 1941, *Terrell Jr. 375* (PH); ♣6 May 1954, *Wherry s.n.* (PH). Delaware Co.: 30 Apr 1892, *Brinton M. D.* (PH-3 sheets); Apr 1903, *Conard s.n.* (PH); 5 Apr 1935, *Fogg Jr. 7996* (PH); 1 May 1904, *Jahn s.n.* (PH); 4 Apr 1946, *Proctor 1716* (NHA). Northampton Co.: 2 May 1969, *Tucker s.n.* (DOV). Philadelphia Co.: ♣20 Apr 1921, *Henslow s.n.* (PH); ♣23 Apr 1932, *Hermann 2752* (NA); ♣May 1878, *Martindale s.n.* (NA, LGO); ♣12 May 1904, *Van Pelt s.n.* (PH). WASHINGTON. King Co.: 8 Mar 2000, *Zika & Jacobson 14827* (WTU). WEST VIRGINIA. Wood Co.: 12 Apr 2003, *Grafton s.n.* (WVA).

Ranunculus ficaria L. subsp. **ficariiformis** (F.W. Schwartz) Rouy & Fouc
U.S.A. MISSOURI. Saint Louis Co.: 19 May 1994, *Ladd 18515* (MO). **NEW YORK. Bronx Co.:** 17 Apr 1988, *Mori & Gracie 18815* (GH, MO, NY). **Nassau Co.:** 8 May 1950, *Abbott s.n.* (CU). **Suffolk Co.:** 1 May 2003, *Glenn 8215* (BKL). **Westchester Co.:** 2 May 1994, *Walker 684* (NY). **NORTH CAROLINA. Wake Co.:** 11 Apr 2005, *Krings 1271* (AUA, F, FLAS, GA, LSU, MISS, NCSC-2 sheets, NCU, TEX, UNA, US, USF, USCH, VDB). **OHIO. Butler Co.:** 5 Apr 1988, *Cusick 27224* (OS). **PENNSYLVANIA. Delaware Co.:** 16 May

1915, *Cramfondi* s.n.* (PH); 26 May 1920, *Long 23006* (PH). **Philadelphia Co.:** 7 May 1910, *St. John 111* (GH). **TEXAS. Tarrant Co.:** 8 Mar 2008, *Nesom FW08-1* (NCSC).

Ranunculus ficaria L. (subspecific determination not feasible)
U.S.A. CONNETICUT. Middlesex Co.: 27 Apr 1991, *Swan s.n.* (Y). **New Haven Co.:** 18 Apr 1995, *Brown 1* (Y); 10 May 1993, *Souther s.n.* (Y). **MARYLAND. Baltimore Co.:** 25 Apr 1948, *Moudry s.n.* (MARY); 21 Apr 1968, *Redman s.n.* (BALT). **Howard Co.:** 17 Apr 1966, *Engh s.n.* (MARY). **Prince Georges Co.:** 19 Apr 1979, *Mills Jr. 14* (BRIT). **MASSACHUSETTS. Barnstable Co.:** 27 Apr 2001, *Mehrhoff 21129* (CONN). **MICHIGAN. Ingham Co.:** 10 Apr 1991, *Stephenson s.n.* (MSC). **NEW JERSEY. Union Co.:** 3 Apr 1977, *Moldenke & Moldenke 31285* (NY); 10 May 1978, *Moldenke & Moldenke 31642* (NY). **NEW YORK. Nassau Co.:** 4 May 2004, *Bennett 030031* (BKL). **Queens Co.:** 27 Apr 1918, *Ferguson 7* (NY); 17 Apr 1921, *Martin s.n.* (NY); 30 Apr 1876, *Schrenck s.n.* (CU). **OHIO. MONTGOMERY Co.:** 18 Mar 1990, *McCormac 1977* (MICH, OS). **OREGON. Multnomah Co.:** 4 Apr 1991, *Zika 11066* (OSC). **PENNSYLVANIA. Chester Co.:** 15 Apr 1957, *Wilkens 9129* (PH). **Delaware Co.:** 7 May 1977, *Brown 199* (Y); 24 Apr 1894, *MacElwee Jr. s.n.* (PH). **Philadelphia Co.:** 11 Apr 1954, *Fogg Jr. 21461* (PH). **WEST VIRGINIA. Monongalia Co.:** 12 Apr 1995, *Baer s.n.* (WVA). **Ritchie Co.:** 25 Apr 1971, *Elliott s.n.* (WVA); 27 Apr 1991, *Grafton s.n.* (WVA).

ACKNOWLEDGMENTS

We thank the following herbaria and their staff for making specimens available to us for study: A, AUA, BALT, BH, BKL, BRIT, CONN, CU, DOV, F, FLAS, GA, GH, HNH, ILLS, KE, LGO, LSU, MARY, MASS, MICH, MISS, MO, MOR, MSC, MT, MU, NA, NCSC, NCU, NHA, NY, OS, OSC, PH, POM, TENN, TEX, UNA, US, USF, USCH, VDB, VPI, WTU, WVA, Y. We also thank David Schuman and Steve Stanislav for statistical support. Three anonymous reviewers offered constructive comments on an earlier draft of the ms.

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